SUPPLEMENTARY TABLE AND FIGURE LEGEND

Supplementary Table 1 A summary of FKF1 NLOV mutagenesis

Supplementary Table 2 Comparison of the light-inducible systems

Supplementary Figure 1 Mutant screens of FKF1 under dark.

(**A**) A summary of FKF1 (NLOV) mutagenesis (n=160). (**B**) Schematic representation of mutant tests using NLOV-VP16 and GI-Gal4DBD constructs in the dark. (**C**) A summary of Luc assay using 81 mutant candidates under the dark conditions. Forty five samples (red numbers) showed low background (n=1-2). The G128D mutant (red column and dashed line) and wild-type (WT, green) were used as controls. #120 was an empty vector (pc3, pcDNA3).

Supplementary Figure 2 Developing an illumination system in culture incubator.

A block diagram (**A**) and a sketch (**B**) of our blue LED illuminator are shown. The illuminator was installed into a CO_2 incubator (**C**) that enables precise control of temperature, CO_2 and blue LED illumination (**D**), 447.5 nm, 0.5 mW).

Supplementary Figure 3 Mutant screens of FKF1 with illumination.

A summary of the Luc assay results testing the 45 candidates that showed low background under dark conditions in Supplementary Figure 1C. Six mutants (#52, 90, 117, 119, 143 and 154) demonstrated similar or higher Luc induction with light compared to G128D (n=1-2). MT, a light-insensitive mutant. The result of the six candidates after repeated tests was shown in Figure 1C (inset) with statistical analyses. Supplementary Figure 3 inset delineates the illumination protocol used in these experiments (blue LED, 447.5nm, 0.5 mW, 6.25 $\mu W/mm^2$).

Supplementary Figure 4 Characterization of NLOV/GI-based systems using Western blot.

Western blotting images of Gal4DBD-NLOV (G128D or H105L) (A) and the full-length and deleted GI fragments (1-1,173, 960, 673, 563 and 391aa) fused with Gal4DBD at the C-terminus (B) and the shorter fragments of GI (1-100, 391, 563 and 673aa) fused with Gal4DBD (C). For Supplementary Figure 4 A-C, the arrowheads indicate the western blot bands for the various forms of Gal4DBD. (**D**) Western blotting images of FLAG-tagged GI-VP16.

Supplementary Figure 5 Examining the effects of FLAG-tag on the FKF1/GI-based system.

(**A**) The illumination protocol used is shown (blue LED, 447.5nm, 0.5 mW, 6.25 $\mu W/mm^2$). (**B**) Luciferase assay to examine the effect of a FLAG-tag addition on GI-VP16 with Gal4DBD-NLOV (G128D)-HA-tag response to illumination (n.s., not significant n=6-9 in 2 independent experiments, mean ± s.e.m.).

Supplementary Figure 6 Comparison of the FKF1/GI-based system to the CRY2/CIB1-based LITE2.0 system to control transcription with light.

(**A**) The illumination protocol that was used (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm^2). (**B**) The short fragments of CRY2 (CRY2PHR) and modified CIB1 were used in the LITE2.0 system (ref. 4). (**C**) Luc assay to test the FKF1 (NLOV, H105L)/GI-based system and LITE2.0. The LITE2.0 system demonstrated significantly increased dark signal compared to the NLOV/GI-based system (** P<0.01, n=8-10 in 4 independent experiments, mean ± s.d.). (**D**) Fluorescent images using the NLOV/GI-based and LITE2.0 systems in HEK 293T cells to express red fluorescent proteins (RFP) in live cells. Hoechst 33285 was used for nuclear staining. Scale bar, 20 μm. (**E**) Western blotting using the NLOV/GI-based and LITE2.0 systems to express destabilized green fluorescent protein (dsGFP) in HEK 293T cells. A housekeeping molecule, β-tubulin, was examined as an internal control in the cells. D, dark. L, light. (**F**) Quantification of dsGFP proteins to compare the NLOV/GI-based and LITE2.0 systems. The expression of dsGFP was normalized to β-tubulin expression (n.s., not significant ** P<0.01 n=4 in two independent experiments, mean ± s.d.). The LITE2.0 system demonstrated significantly increased dark signal when compared to the FKF1/GI-based system.

Supplementary Figure 7 Comparisons of the FKF1/GI-based system to the other light-inducible systems.

Comparisons of the FKF1/GI-based systems to the other light-inducible systems, the VVD/LightOn- and EL222-based systems (n=4-16, mean ± s.d.). The DNA amounts indicated were used for transfection in a well of 24-well plates. Illumination protocol used is described in the insert (Protocol B).

Supplementary Figure 8 Comparison and optimization of the FKF1/GI and Cry2/CIB1 system.

(**A**) Illumination protocol used in Figure S8B is indicated (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm^2). (**B**) Comparison of NLOV/GI and 1:1 DNA ratio CRY2/CIB1 (n=4 mean ± s.d.). (**C**) Illumination protocol used for in Figure S8D,E,H and I is indicated (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm^2). (**D, E**) DNA ratio optimization of NLOV/GI and CRY2/CIBI respectively (* P<0.05, n=3 in 3 independent experiments, mean ± s.d.). CRY2/CIB1 benefits from a ratio of 5:1 CRY2:CIB1. (**F, G**) Illumination protocols used for in Figure S8H and I are indicated (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm^2). (**H, I**) Luciferase assay results of NLOV/GI and CRY2/CIB1 respectively with variable illumination cycles. (n.s., not significant, ** P<0.01, *** P<0.001, n=12 in 2 independent experiments for Protocol B 8s and Protocol B 2s, n=27 and n=58 for CRY2 and FKF1 Protocol B respectively as compiled Legacy data was used, mean ± s.d.). NLOV/GI has a significantly decreased induction when exposed to the 2s protocol. CRY2/CIB1 has a significantly decreased induction when exposed to shorter light and longer dark cycles as compared to Protocol B.

Supplementary Figure 9 Representative FACS images and NIH 3T3 FACS results

(**A-C**) Representative FACS images for HEK 293T, Human Fibroblasts and NIH 3T3 cells respectively. The raw FACS data was sorted for live cells, then the mCherry+ (transfected cells) were selected. Finally, the GFP^+ cells were selected for in order to yield the GFP positive population of transfected cells. (**D**) A comparison of NLOV/GI and CRY2/CIB1 systems transfected into NIH3T3 cells illuminated following protocol B with Gal4-UAS-dsGFP (*** P<0.001, n=8-12 in 2 independent experiments, mean ± s.d.). mCherry (red fluorescent protein, RFP) plasmid was used as transfection control.

Supplementary Figure 10 Cry2-VP16 and CIB1 NLOV/GI in vivo application, H105L with VP16, VP64 and NLOV/GI H105L G128D and NLOV homology model

(**A**) Representative images of mouse livers from mice after illumination (dark (top), light (bottom) using Gal4-UAS-GFP with CRY2-VP16 and CIB1-Gal4DBD with hydrodynamic tail vein plasmid injection method (n=4). Scale bar, 2mm. (**B**) Homology model of FKF1 NLOV domain highlighting the H105 (red), G128 (purple) amino acids and the FKF1 family conserved loop (green). (**C**) Illumination protocol used in Supplementary Figure 10D and E is indicated (blue LED, 447.5nm, 0.5 mW, 6.25 $\mu W/mm^2$). (**D**) Luciferase assay results of NLOV/GI H105L and the NLOV/GI H105L G128D double mutant. (*** P<0.001, n=8 in 2 independent experiments, mean ± s.d.). The double mutant has a significantly reduced induction amount compared with the NLOV/GI H105L. (**E**) Luciferase assay results of NLOV with GI-VP16 and GI-VP64. (n.s., not significant, n=12 in 2 independent experiments, mean ± s.d.). Using protocol B, there is no difference between the use of VP16 or VP64.

Supplementary Figure 11 The application of the IRES version of FKF1/GI-based light-induced transcription system for Myod1 expression

(**A**) Luciferase assay results of HEK 293T cells with co-transfection of two plasmids: Gal4DBD-NLOV (H105L)-HA (G4D-NLOV) and FLAG-GI-VP16 (GI-VP16) and single transfection using the 2A self-cleavage and internal ribosome entry site (IRES) systems (*** P<0.001, n=8 in 2 independent experiments, mean ± s.d.). (**B**) Luciferase assay results of mouse embryonic fibroblasts (MEF) with the new IRES construct: FLAG-GI-VP16-IRES-Gal4DBD-NLOV (H105L)-HA and Gal4 UAS firefly luciferase (n=9 in 2 independent experiments, mean ± s.d.). (**C**) Quantification of *Myod1* transcript expression in MEF with the new IRES construct: FLAG-GI-VP16-IRES-Gal4DBD-NLOV (H105L)-HA and Gal4 UAS-Myod1 (n=7 in 2 independent experiments, mean ± s.d.). (**D**) Representative fluorescent images of immunocytochemistry for Myod1 protein expression in illuminated MEF with FLAG-GI-VP16-IRES-Gal4DBD-NLOV (H105L)-HA and Gal4 UAS-Myod1. Hoechst 33285 (blue) was used for nuclear staining. Anti

mouse Myod1 antibody was used for Myod1 staining with Alexa 488 (green) secondary antibody. Scale bar, 50 μm. Protocol B was used in these experiments.

SUPPLEMENTARY NOTE

LED illuminator development:

The blue LED illuminator with heat control and a built-in fan was designed and developed by J. Jung.

The illuminator has following expandable features:

1) Switch on/off (automatically/manual) is available using a LED assembly consisting of 7 rebel LEDs (Luxeion, #SR-02-R0500). The intensity/brightness of the LED can be adjusted from 0% (off) to 100% (max brightness).

2) Typical characteristics of one LED Assembly is 6W, fixed wavelength 447.5nm (other assemblies are also available).

3) While the LEDs are turned on, thermal heat will be sank into the water-jacked incubator in which the LED assembly is mounted.

4) The fan mounted above the heat sinks can be turned on (automatically or manually) to enforce the airflow through the heat sinks to ensure a homogeneous temperature below the LEDs.

5) While the LEDs are turned off, heat elements can be switched on (automatic/manual). These elements should emit the same amount of thermal heat as when the LEF's are turned on. This would help stabilize the temperature in the incubator.

6) Small, enclosed high precision temperature sensors (+/- 0.065 °C resolution) are used to monitor temperature of the sample and control areas below the LEDs. Temperature data are logged with time/date stamps and stored on an SD card. Data is also shown on a display.

7) A programmed sequence (turn the LED/heater/fan on and off, measure the temperature and log the data) can last several days and is started by pushing the start button on the controller. The time sequence limit is set by the software (Arduino).

8) Interval length adjustments and LED intensity settings are determined (0.5 mW, 6.25 $\mu W/mm^2$) by changing the appropriate parameters in the software, which can be easily updated when connecting the micro controller to a computer using the USB interface.

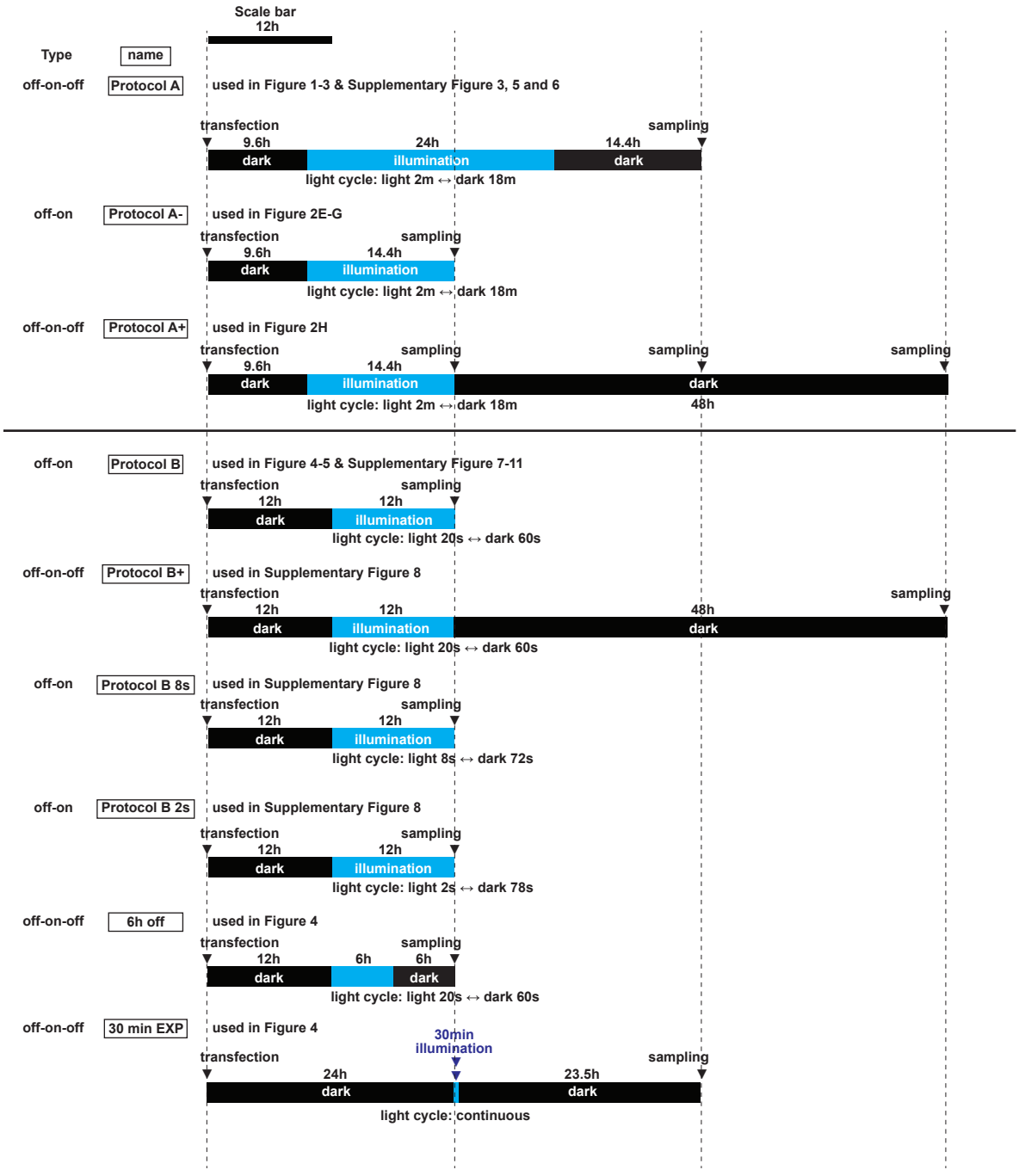
EL222 transfection:

Following Dr. Motta-Mena's protocol and advices, we transfected the EL222 system in HEK 293T cells using the following ratio of plasmids: VP-EL222: pGL-C120-Luc: HSV-TK-Renilla = 5: 1: 0.04 (total plasmids DNA, 0.5 µg/well in 24-well plate).

Statistical Analyses:

Figure	**Statistics used**
Figure 1C	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure S3	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure 2B, C	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure 2E, F	F Test and Unpaired T test
Figure 2G, H	F Test and Unpaired T test
Figure S5B	F Test and Unpaired T test
Figure S6C, F	F Test and Unpaired T test
Figure 3	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure S7	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure 4C	F Test and Unpaired T test
Figure 4H	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure 4G	F Test and Unpaired t test with Welch's correction
Figure 4I-K	F Test and Unpaired t test
Figure 4N-O	Bartlett test and One way Anova with Dunnett's Multiple comparisons test
Figure S7B	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure S7 D, E, I, J	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure S9D	F Test and Unpaired T test with Welch's correction
Figure 5D	F Test and Unpaired t test with Welch's correction
Figure S10 B	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure SF11 A	Bartlett test and one-factor ANOVA with a Bonferroni/Dunn test
Figure SF11 B	F Test and Unpaired T test

Protocol summary for illumination



Plasmid Summary:

pcDNA3-NLS-Gal4DBD-dFKF1(N-LOV, H105L)-HA v.2 (clone #13)

(Cloning) KpnI

Kozak

(ATG): Start

Red: NLS

Blue: Gal4 DNA binding domain (Gal4DBD)

NotI

Red: dFKF1(N-LOV, H105L)

gct: linker

Blue: HA-tag, TACCCATACGATGTTCCAGATTACGCT

(TAG): STOP

(Cloning) XbaI

(sequence)

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pcDNA3-FLAG-GI-VP16 (clone #4):

(Cloning) EcoRI

Kozak

(ATG): Start

FLAG-tag

Red: GI

NotI & linker (agtgctggt, SAG)

Blue: VP16

(PCR template: F-type VP16 activation domain in pTet-On Advanced plasmid)

(TAA): STOP

(Cloning) XbaI

(sequence)

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pcDNA3-CRY2-VP16

(Cloning) KpnI

Kozak

(ATG): Start

Red: CRY2

NotI & linker (agtgctggt, SAG)

Blue: VP16

(TAA): STOP

(Cloning) XbaI

(sequence)

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pcDNA3-CIB1-Gal4DBD

(Cloning) KpnI

Kozak

(ATG): Start

Red: CIB1

NotI & linker (agtgctggt, SAG)

Blue: Gal4DBD

(TAA): STOP

(Cloning) XbaI

(sequence)

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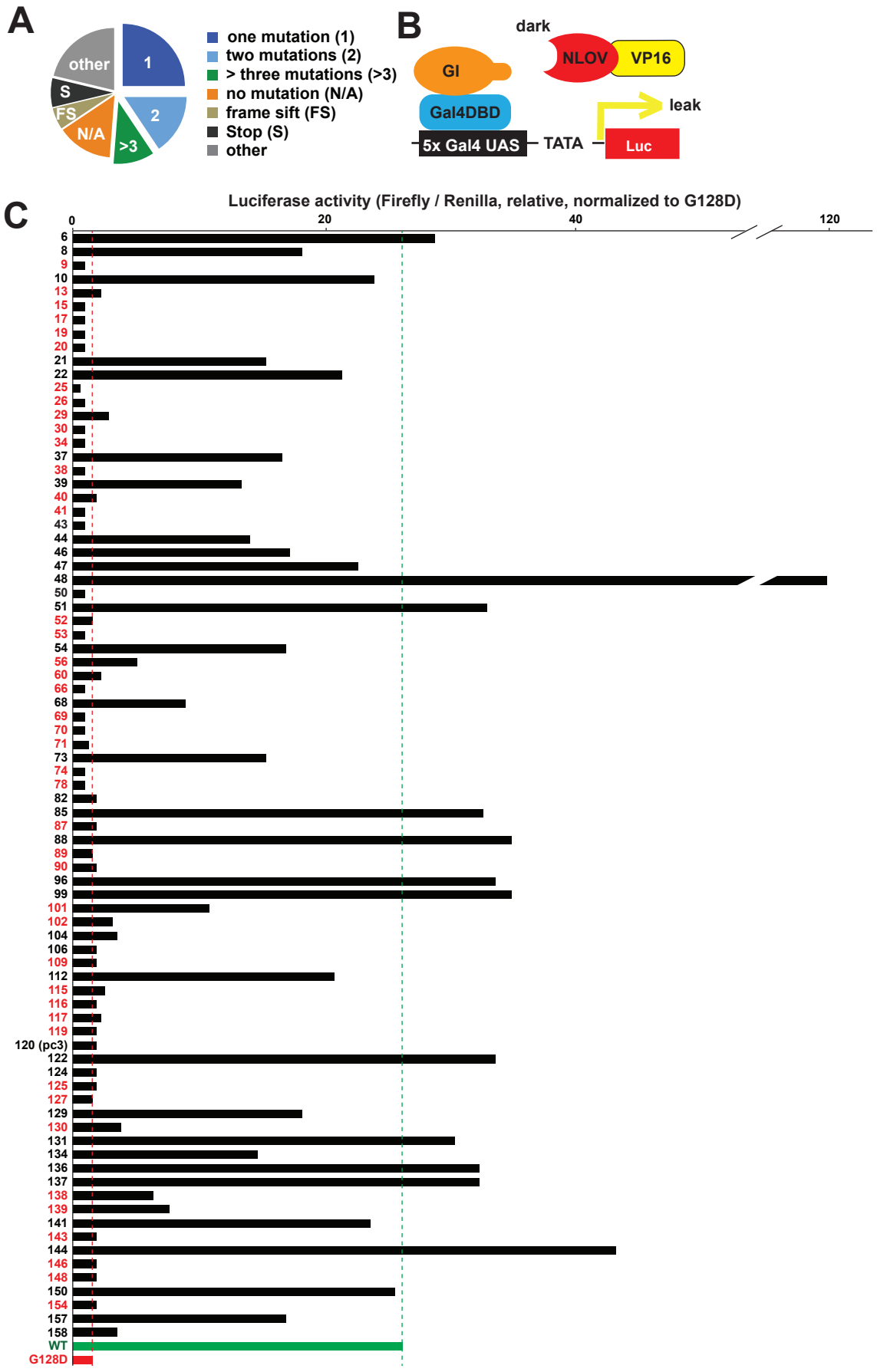
Supplementary Table 1 A summary of FKF1 NLOV mutagenesis

No.	1st test	2nd test	mutation(s)					No.	1st test	2nd test	mutation(s)					
1			no					81			x					
2			G45A	K135stop				82			V74D					
3			no					83			frame shift					
4			x					84			x					
5			G18S	R134I	Q163stop			85			I149V					
6			K16I					86			x					
7			x					87			I7T	D65Y	T155S			
8			T155I					88			Y96C					
9			I69F					89			V20I	L62P	D152V			
10			L130I					90			P54H	H105L				
11			L140stop					91			x					
12			x					92			no					
13			A2T	G30V				93			no					
14			no					94			no					
15			F93Y	R97S				95			no					
16			x					96			C27S					
17			E63V	Y96N	H105Y	E121G	K135N	97			x					
18			R134stop					98			x					
19			I57F					99			Q34H	I69V				
20			Y49H	N132S				100			x					
21			E122Q					101			R82P					
22			R17S					102			V113A					
23			E63stop					103			no					
24			x					104			R19K					
25			S55P	P106Q				105			V113A					
26			K16R	F126V				106			L146H					
27			no					107			x					
28			G8stop					108			no					
29			E38K	A83V				109			K39E	T79N				
30			I57N	F75L	D152H			110			K13STOP					
31			no					111			x					
32			x					112			M46T	D152N				
33			x					113			no					
34			E36V	I57F				114			x					
35			no					115			I7F	V71D	F93I	N142K		
36			R97stop					116			R3S	A61V	E125K			
37			V113I					117			K135T					
38			E76V	N90I	P99H			118			R14G	Y96STOP				
39			T138A					119			H105R	R145Q				
40			E33A	Y70F	I116M			120			x					
41			L146H					121			L68STOP					
42			x					122			I124V					
43			V86L					123			no					
44			V44D					124			H5R	E21D	I57F	P67H	F75I	G123D
45			no					125			F47Y	E76V				
46			K15R					126			no					
47			R143K					127			F75I	P139H				
48			K39M	F56I				128			no					
49			x					129			E129G					
50			E21D	P50T	R145G	D152V		130			Y49F	F165L				
51			E43D					131			R117K					
52			P54H	G137C				132			x					
53			F66Y	D98E				133			no					
54			I31T					134			K15R	L62Q	Q102H			
55			x					135			frame shift					
56			D152V					136			E38G					
57			x					137			V20A	V35E				
58			x					138			G45W					
59			x					139			E22V	L120I				
60			R118G					140			x					
61			x HA tag stop					141			E24K					
62			frame shift					142			x					
63			frame shift					143			I31N	D60E	E125D			
64			x					144			E43D					
65			x					145			E22G	C27F	Q34L	C119STOP	E122D	
66			A10T	R19I	E25G	F47L	E76V	146			E38D	F93Y	R117G	R145Q		
67			x					147			no					
68			P54L					148			F93Y	F126I				
69			R14L	P67L	D136V			149			no					
70			M51V	L140S				150			I7F					
71			D109V					151			E21STOP	V77A	L94I	L144P	H158	
72			x					152			no					
73			K16I	M46L				153			no					
74			Y70N					154			N28Y	V112G				
75			x					155			x					
76			x					156			x					
77			frame shift					157			D84E					
78			A1V	Y81H	D84Y	E85G		158			D84G	R150C	S166Y			
79			no					159			frame shift					
80			x					160			x					

low background
slightly low background
higher background
x: sequencing failed or no insertion
no: no mutations
the same mutations exist in other mutants
Further tests with illuminaiton

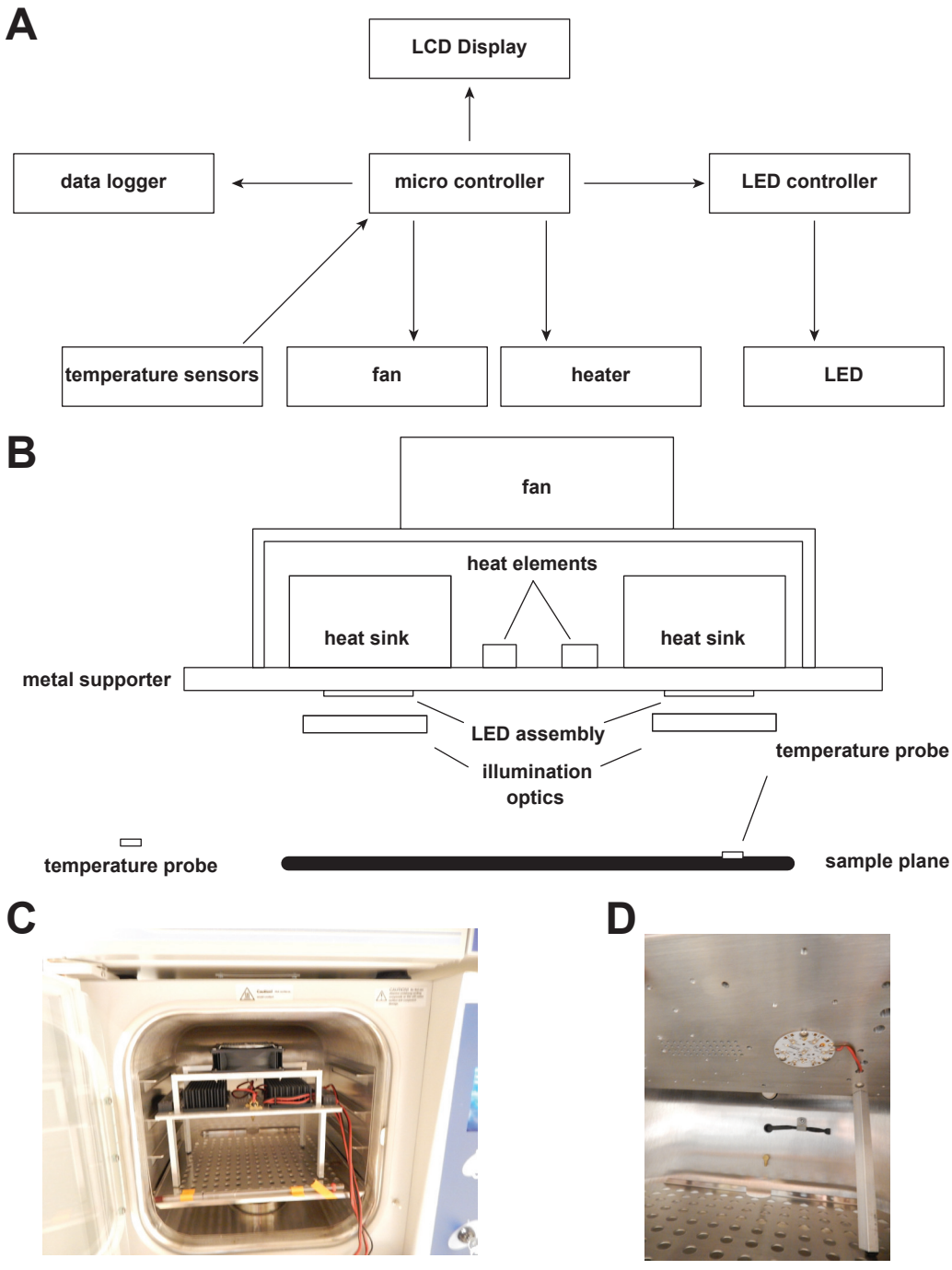
Supplementary Table 2 Comparison of the light-inducible systems

	NLOV (H105L)/GI	CRY2/CIB1	VVD (LightOn)	EL222
dark (Firefly/Renilla, mean ± s.d.)	0.0278 ± 0.0049	0.5673 ± 0.1308	9.2291 ± 1.0259	0.1019 ± 0.0075
dark (relative, normalized to NLOV dark)	1.0	20.4	332.0	3.7
light (Firefly/Renilla, mean ± s.d.)	2.5369 ± 0.4886	56.3632 ± 12.2315	162.2991 ± 29.8400	1.1729 ± 0.1783
light (relative, normalized to NLOV dark)	91	2,027	5,838	42
fold-induction with light	91	99	17.6	11.5
	ratio 1:1	ratio 5:1		



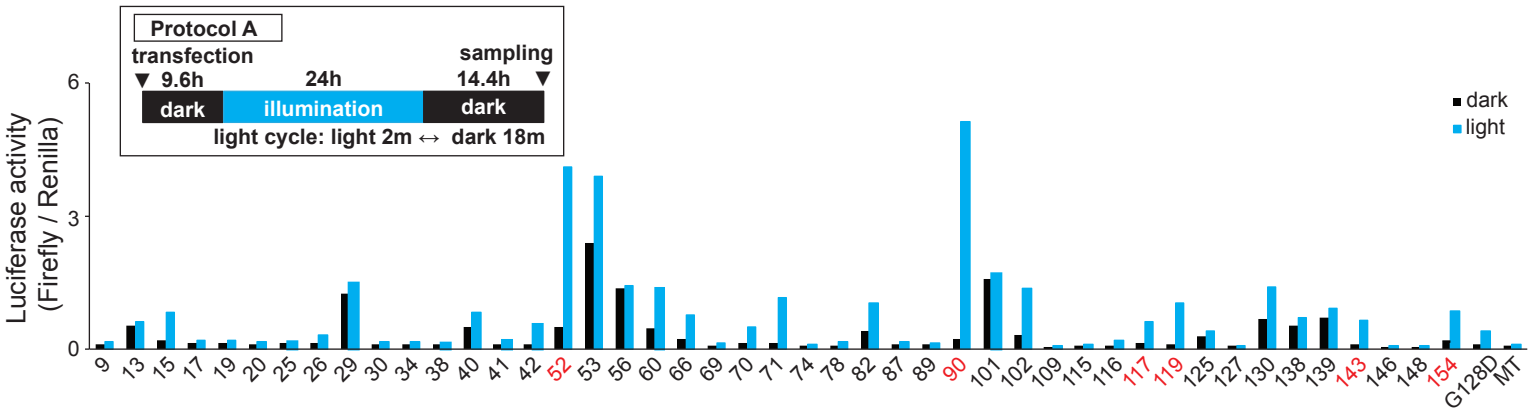
Supplementary Figure 1 Mutant screens of FKF1 under dark.

(**A**) A summary of FKF1 (NLOV) mutagenesis (*n*=160). (**B**) Schematic representation of mutant tests using NLOV-VP16 and GI-Gal4DBD constructs in the dark. (**C**) A summary of Luc assay using 81 mutant candidates under the dark conditions. Forty five samples (red numbers) showed low background (*n*=1-2). The G128D mutant (red column and dashed line) and wild-type (WT, green) were used as controls. #120 was an empty vector (pc3, pcDNA3).



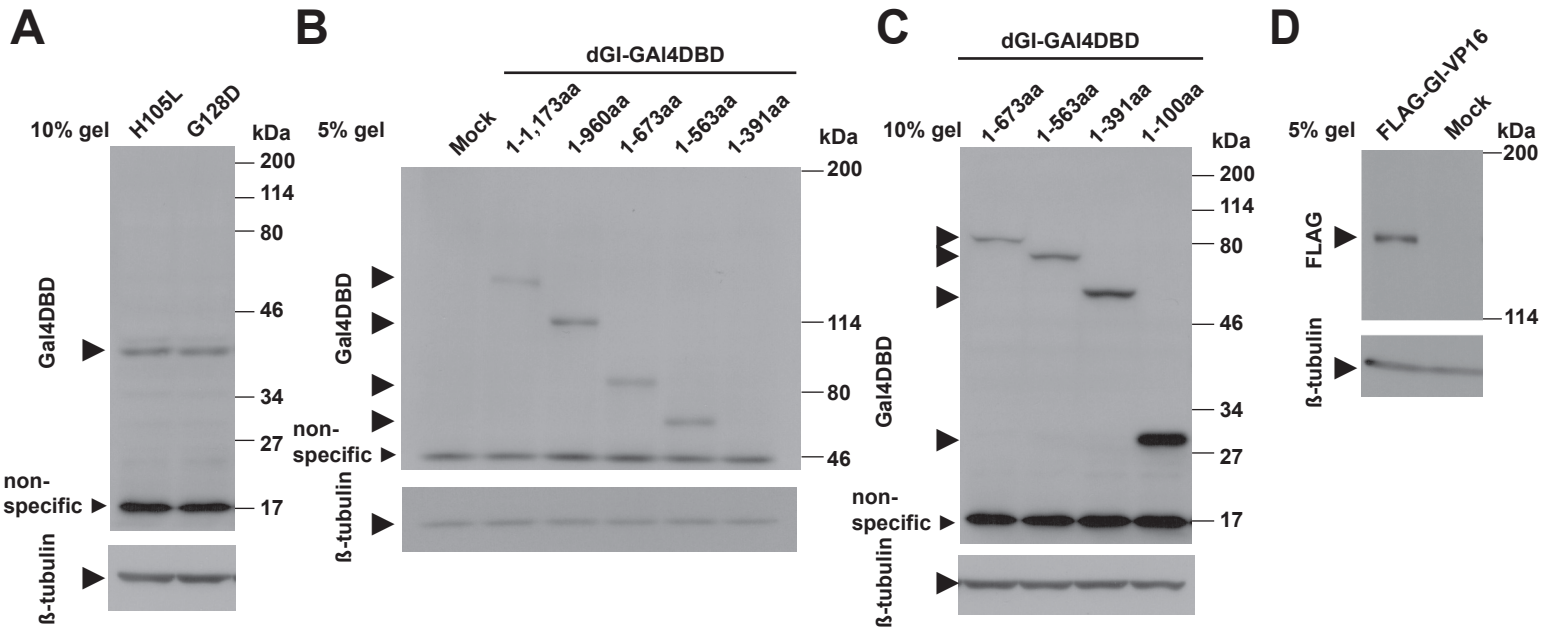
Supplementary Figure 2 Developing an illumination system in culture incubator.

A block diagram (**A**) and a sketch (**B**) of our blue LED illuminator are shown. The illuminator was installed into a CO_2 incubator (**C**) that enables precise control of temperature, CO_2 and blue LED illumination (**D**), 447.5 nm, 0.5 mW).



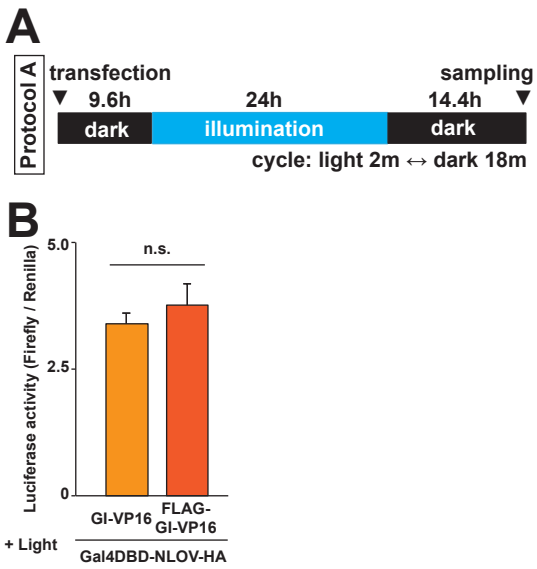
Supplementary Figure 3 Mutant screens of FKF1 with illumination.

A summary of the Luc assay results testing the 45 candidates that showed low background under dark conditions in Supplementary Figure 1C. Six mutants (#52, 90, 117, 119, 143 and 154) demonstrated similar or higher Luc induction with light compared to G128D (*n*=1-2). MT, a light-insensitive mutant. The result of the six candidates after repeated tests was shown in Figure 1C (inset) with statistical analyses. Supplementary Figure 3 inset delineates the illumination protocol used in these experiments (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm^2).



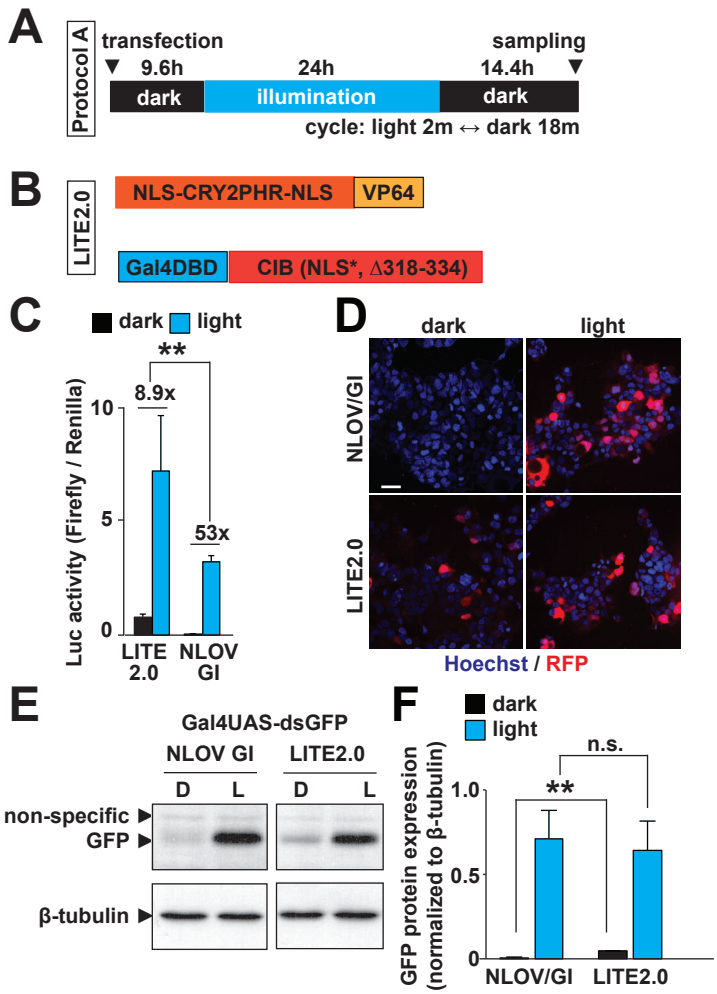
Supplementary Figure 4 Characterization of NLOV/GI-based systems using Western blot.

Western blotting images of Gal4DBD-NLOV (G128D or H105L) (**A**) and the full-length and deleted GI fragments (1-1,173, 960, 673, 563 and 391aa) fused with Gal4DBD at the C-terminus (**B**) and the shorter fragments of GI (1-100, 391, 563 and 673aa) fused with Gal4DBD (**C**). For Supplementary Figure 4 A-C, the arrowheads indicate the western blot bands for the various forms of Gal4DBD. (**D**) Western blotting images of FLAG-tagged GI-VP16.



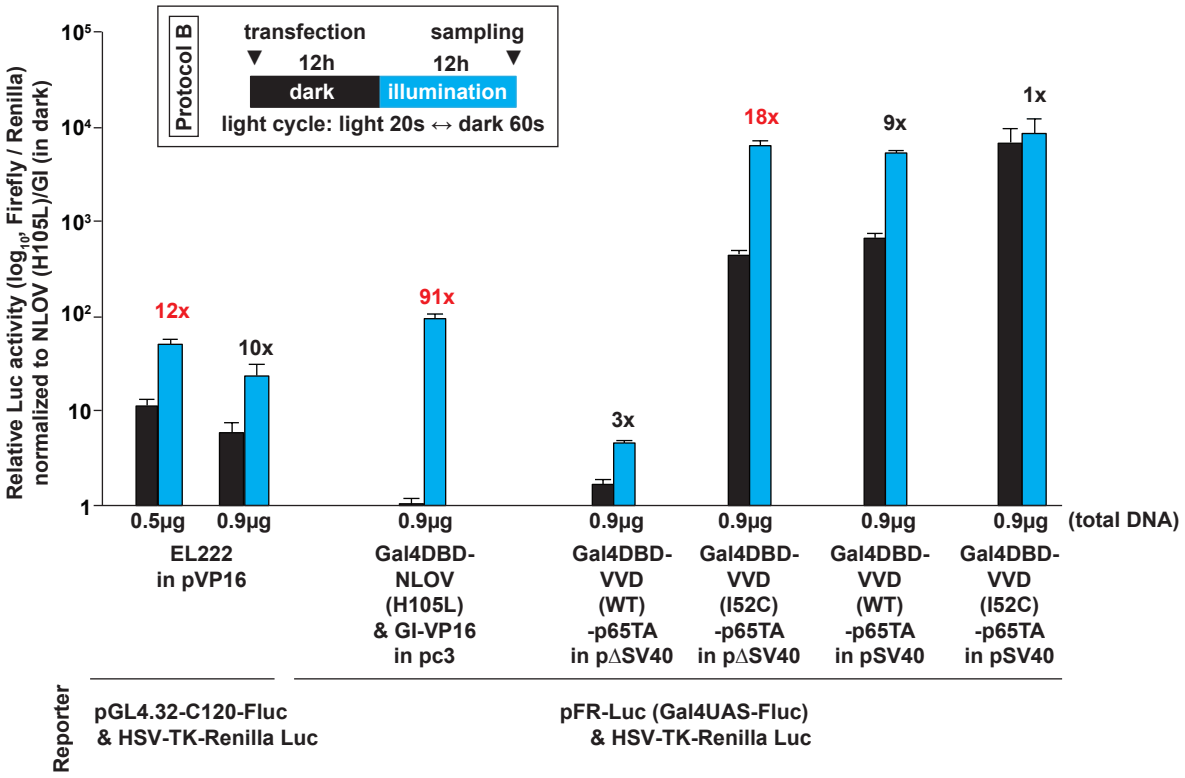
Supplementary Figure 5 Examining the effects of FLAG-tag on the FKF1/GI-based system.

(**A**) The illumination protocol used is shown (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm^2). (**B**) Luciferase assay to examine the effect of a FLAG-tag addition on GI-VP16 with Gal4DBD-NLOV (G128D)-HA-tag response to illumination (n.s., not significant *n*=6-9 in 2 independent experiments, mean ± s.e.m.).



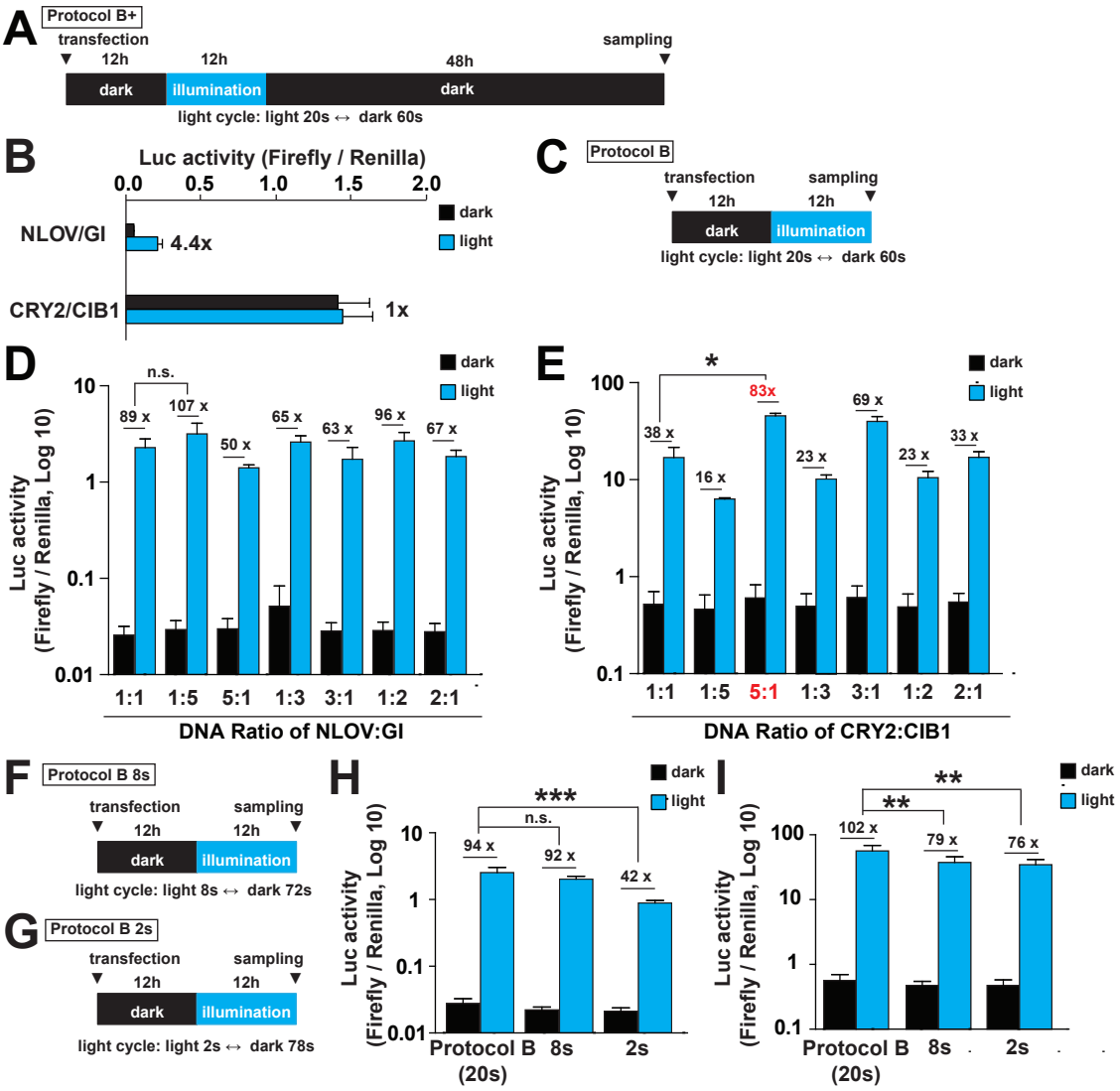
Supplementary Figure 6 Comparison of the FKF1/GI-based system to the CRY2/CIB1-based LITE2.0 system to control transcription with light.

(**A**) The illumination protocol that was used (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm^2). (**B**) The short fragments of CRY2 (CRY2PHR) and modified CIB1 were used in the LITE2.0 system (ref. 4). (**C**) Luc assay to test the FKF1 (NLOV, H105L)/GI-based system and LITE2.0. The LITE2.0 system demonstrated significantly increased dark signal compared to the NLOV/-GI-based system (** P<0.01, n=8-10 in 4 independent experiments, mean ± s.d.). (**D**) Fluorescent images using the NLOV/GI-based and LITE2.0 systems in HEK 293T cells to express red fluorescent proteins (RFP) in live cells. Hoechst 33285 was used for nuclear staining. Scale bar, 20 μm. (**E**) Western blotting using the NLOV/GI-based and LITE2.0 systems to express destabilized green fluorescent protein (dsGFP) in HEK 293T cells. A housekeeping molecule, β-tubulin, was examined as an internal control in the cells. D, dark. L, light. (**F**) Quantification of dsGFP proteins to compare the NLOV/GI-based and LITE2.0 systems. The expression of dsGFP was normalized to β-tubulin expression (n.s., not significant ** P<0.01 n=4 in two independent experiments, mean ± s.d.). The LITE2.0 system demonstrated significantly increased dark signal when compared to the FKF1/GI-based system.



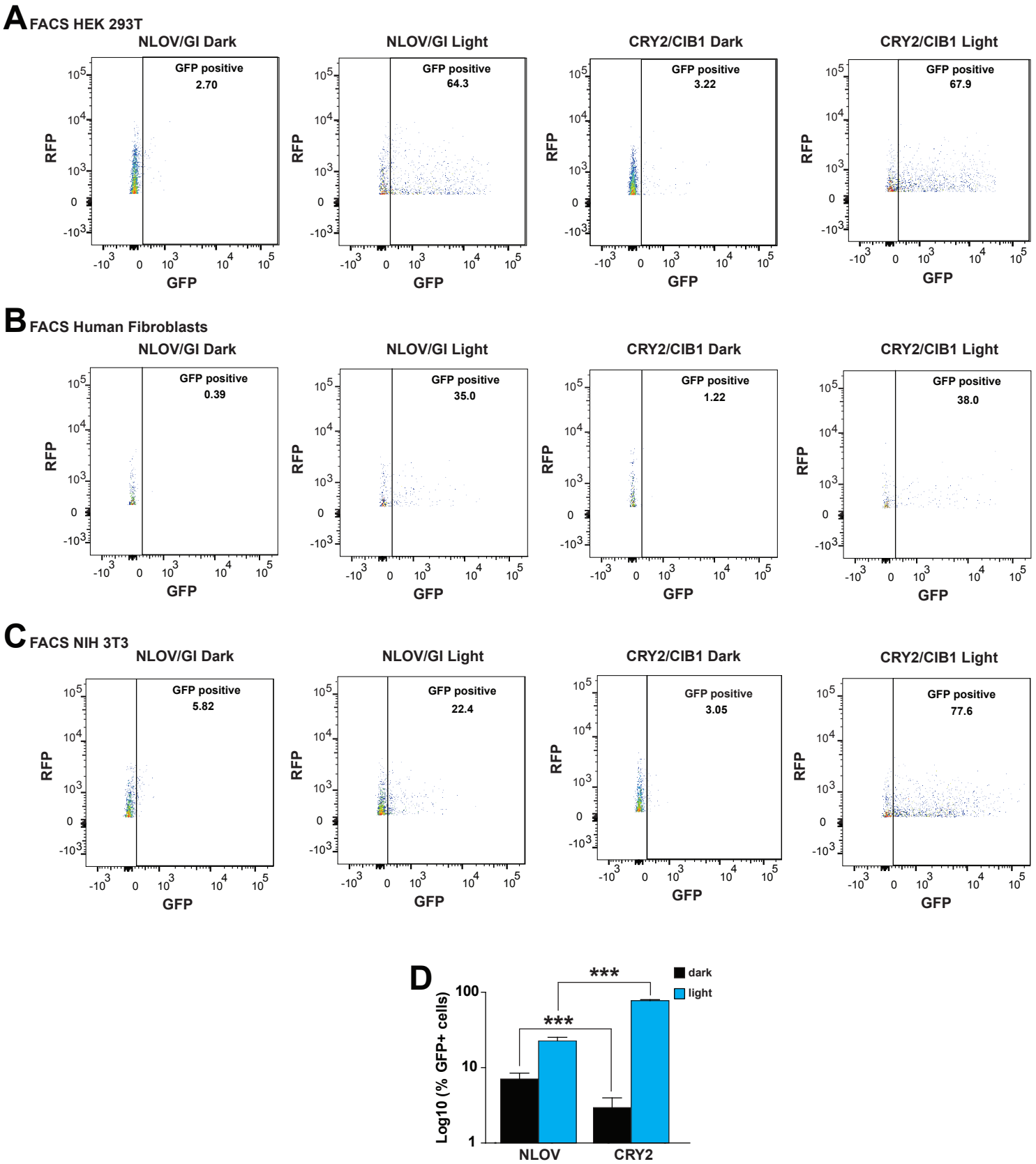
Supplementary Figure 7 Comparisons of the FKF1/GI-based system to the other light-inducible systems.

Comparisons of the FKF1/GI-based systems to the other light-inducible systems, the VVD/LightOn- and EL222-based systems (*n*=4-16, mean ± s.d.). The DNA amounts indicated were used for transfection in a well of 24-well plates. Illumination protocol used is described in the insert (Protocol B).



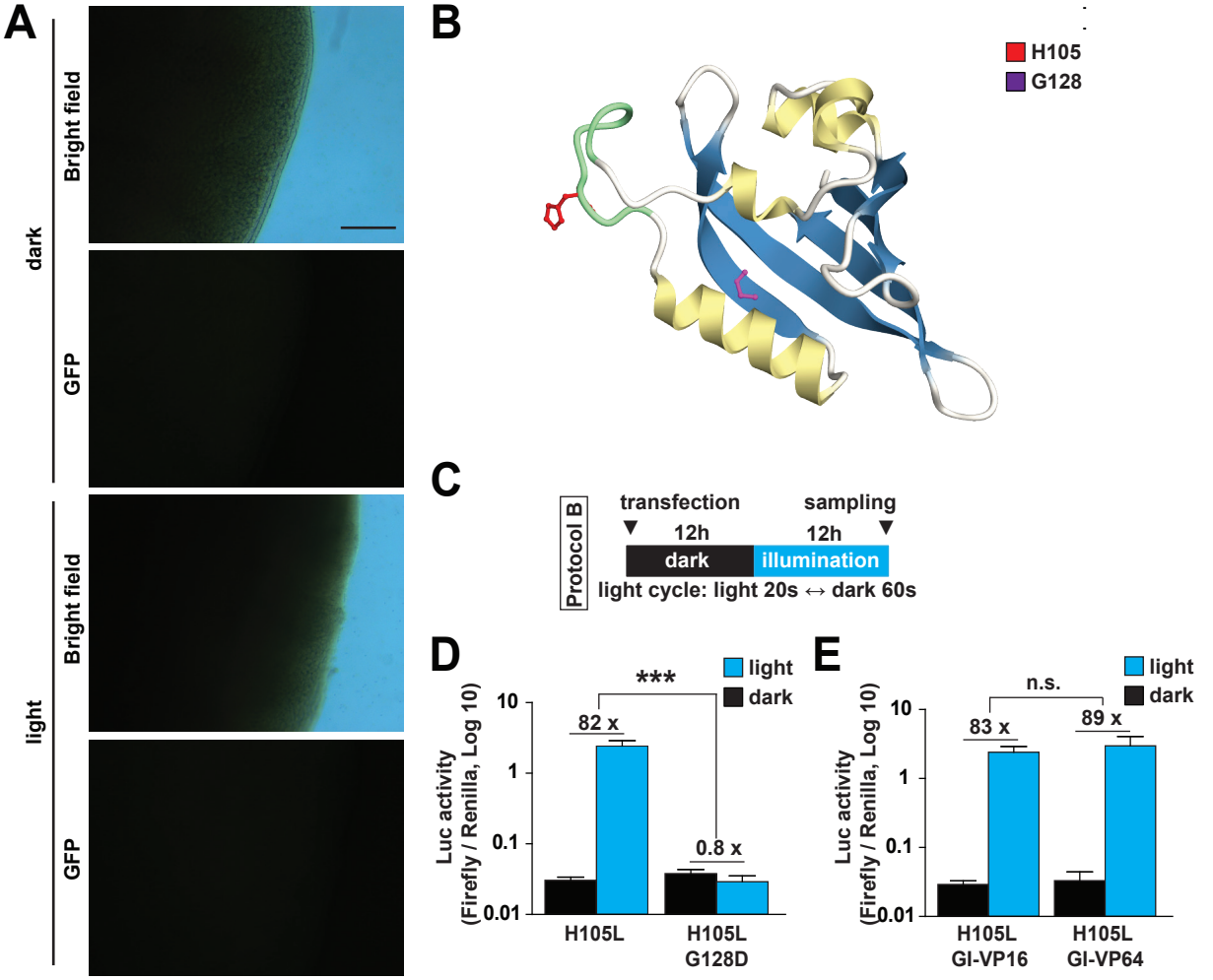
Supplementary Figure 8 Comparison and optimization of the FKF1/GI and Cry2/CIB1 system.

(**A**) Illumination protocol used in Figure S8B is indicated (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm²). (**B**) Comparison of NLOV/GI and 1:1 DNA ratio CRY2/CIB1 (*n*=4 mean ± s.d.). (**C**) Illumination protocol used for in Figure S8D,E,H and I is indicated (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm²). (**D, E**) DNA ratio optimization of NLOV/GI and CRY2/CIBI respectively (* *P*<0.05, *n*=3 in 3 independent experiments , mean ± s.d.). CRY2/CIB1 benefits from a ratio of 5:1 CRY2:CIB1. (**F, G**) Illumination protocols used for in Figure S8H and I are indicated (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm²). (**H, I**) Luciferase assay results of NLOV/GI and CRY2/CIB1 respectively with variable illumination cycles. (n.s., not significant, ** *P*<0.01, *** *P*<0.001, *n*=12 in 2 independent experiments for Protocol B 8s and Protocol B 2s, *n*=27 and *n*=58 for CRY2 and FKF1 Protocol B respectively as compiled Legacy data was used , mean ± s.d.). NLOV/GI has a significantly decreased induction when exposed to the 2s protocol. CRY2/CIB1 has a significantly decreased induction when exposed to shorter light and longer dark cycles as compared to Protocol B.



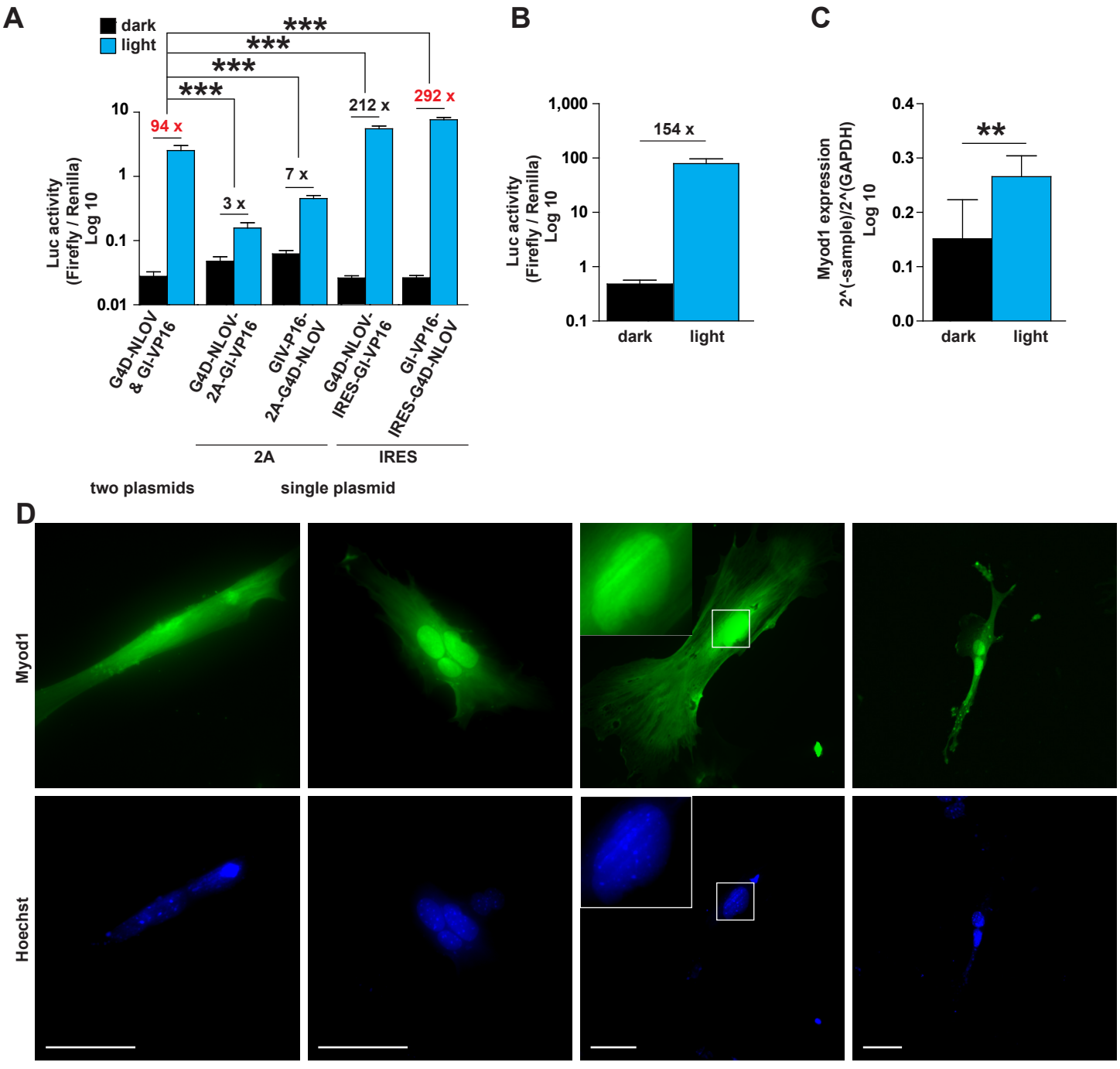
Supplementary Figure 9 Representative FACS images and NIH3T3 FACS results

(**A-C**) Representative FACS images for HEK 293T, Human Fibroblasts and NIH 3T3 cells respectively. The raw FACS data was sorted for live cells, then the mCherry+ (transfected cells) were selected. Finally, the GFP+ cells were selected for in order to yield the GFP positive population of transfected cells. (**D**) A comparison of NLOV/GI and CRY2/CIB1 systems transfected into NIH3T3 cells illuminated following protocol B with Gal4-UAS-dsGFP (*** P<0.001, n=8-12 in 2 independent experiments, mean ± s.d.). mCherry (red fluorescent protein, RFP) plasmid was used as transfection control.



Supplementary Figure 10 Cry2-VP16 and CIB1 NLOV/GI *in vivo* application, H105L with VP16, VP64 and NLOV/GI H105L G128D and NLOV homology model

(**A**) Representative images of mouse livers from mice after illumination (dark (top), light (bottom) using Gal4-UAS-GFP with CRY2-VP16 and CIB1-Gal4DBD with hydrodynamic tail vein plasmid injection method (*n*=4). Scale bar, 2mm. (**B**) Homology model of FKF1 NLOV domain highlighting the H105 (red), G128 (purple) amino acids and the FKF1 family conserved loop (green). (**C**) Illumination protocol used in Supplementary Figure 10D and E is indicated (blue LED, 447.5nm, 0.5 mW, 6.25 μW/mm^2). (**D**) Luciferase assay results of NLOV/GI H105L and the NLOV/GI H105L G128D double mutant. (*** *P*<0.001, *n*=8 in 2 independent experiments, mean±s.d.). The double mutant has a significantly reduced induction amount compared with the NLOV/GI H105L. (**E**) Luciferase assay results of NLOV with GI-VP16 and GI-VP64. (n.s., not significant, *n*=12 in 2 independent experiments, mean ± s.d.). Using protocol B, there is no difference between the use of VP16 or VP64.



Supplementary Figure 11 The application of the IRES version of FKF1/GI-based light-induced transcription system for Myod1 expression

(**A**) Luciferase assay results of HEK 293T cells with co-transfection of two plasmids: Gal4DBD-NLOV (H105L)-HA (G4D-NLOV) and FLAG-GI-VP16 (GI-VP16) and single transfection using the 2A self-cleavage and internal ribosome entry site (IRES) systems (*** P<0.001, n=8 in 2 independent experiments, mean ± s.d.). (**B**) Luciferase assay results of mouse embryonic fibroblasts (MEF) with the new IRES construct: FLAG-GI-VP16-IRES-Gal4DBD-NLOV (H105L)-HA and Gal4 UAS firefly luciferase (n=9 in 2 independent experiments, mean ± s.d.). (**C**) Quantification of Myod1 transcript expression in MEF with the new IRES construct: FLAG-GI-VP16-IRES-Gal4DBD-NLOV (H105L)-HA and Gal4 UAS-Myod1 (**P<0.01 n=7 in 2 independent experiments, mean ± s.d.). (**D**) Representative fluorescent images of immunocytochemistry for Myod1 protein expression in illuminated MEF with FLAG-GI-VP16-IRES-Gal4DBD-NLOV (H105L)-HA and Gal4 UAS-Myod1. Hoechst 33285 (blue) was used for nuclear staining. Anti mouse Myod1 antibody was used for Myod1 staining with Alexa 488 (green) secondary antibody. Scale bar, 50 μm. Protocol B was used in these experiments.